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POTENT CARBOXYLATE INHIBITORS OF STROMELYSIN CONTAINING P2' PIPERAZIC ACIDS AND P1' BIARYL MOEITIES

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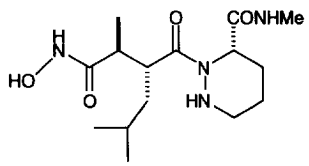
Abstract: Several carboxylate derivatives with variation at the P1' residue were synthesized and evaluated as stromelysin (MMP-3) inhibitors. Compounds containing a biphenyl moiety at P1' were found to be potent inhibitors of MMP-3. An X-ray crystal structure of the most potent compound, carboxylate **19**, revealed an important interaction between the inhibitor's biphenyl and histidine 224 in the S1' pocket of MMP-3.

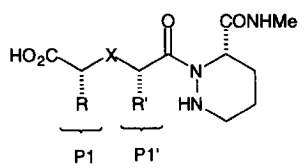
Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are involved in the degradation and remodeling of extracellular matrix and connective tissues.[1] Recent studies have indicated that the uncontrolled overexpression and subsequent proteolytic activities of collagenase (MMP-1) and stromelysin (MMP-3) are increased in patients with rheumatoid and osteoarthritis.[2] MMP-3 appears to play a critical role in cartilage destruction in humans, with enzymatic activity correlating with lession severity in arthritic patients.[3] Stromelysin, therefore, represents a viable therapeutic target for clinical intervention in arthritis.

We recently disclosed the piperazic acid (Ppz) containing hydroxamate **1** as an orally bioavailable, potent inhibitor of MMP-3.[4] As observed in peptides of related interest,[5] the Ppz group was found to impart enhanced water solubility (>15 mg/mL) and oral absorption in comparison to standard proteinogenic amino acids such as Phe and Leu in this position. We were interested in exploring carboxylate analogs of **1** as MMP-3 inhibitors, specifically investigating P1' SAR. The S1' pocket of MMP-3 has been shown to be both deep and hydrophobic,[6,7] and as detailed in this investigation, biphenyl substituents were determined to enhance the potency of analogs containing Ppz in P2'. We describe, herein, the synthesis and evaluation of inhibitors having the general formula **2**.



1

MMP-3 IC_{50} = 30 nM



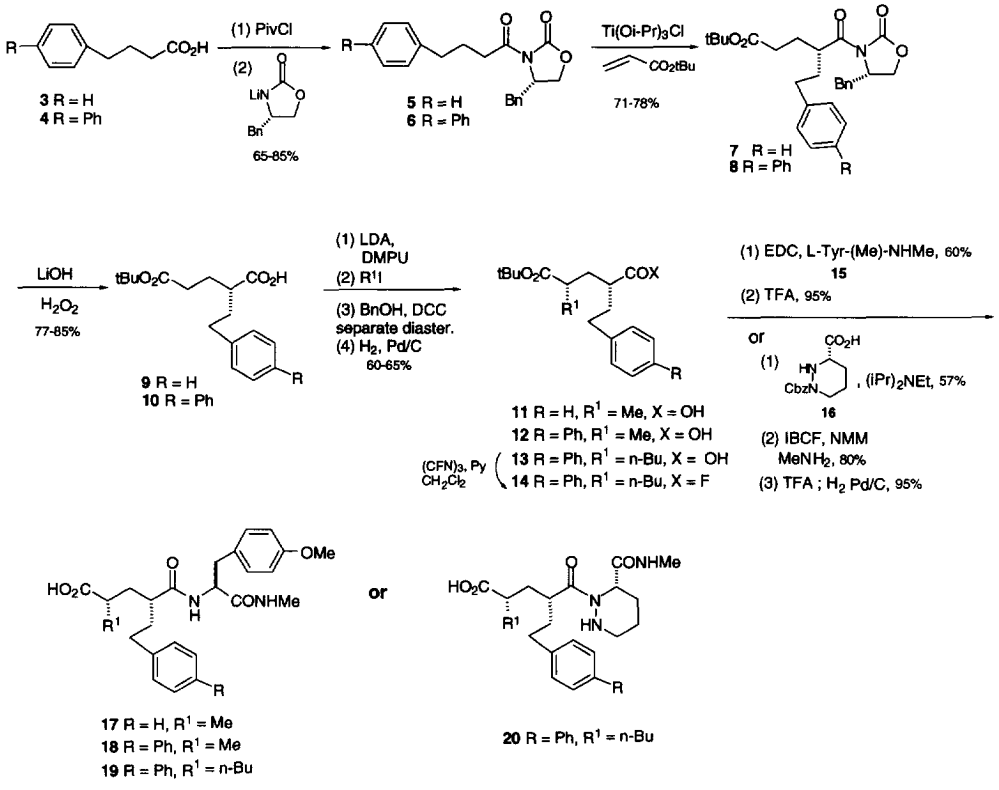
2 (X = CH_2, NH)

Synthesis

The C-carboxyalkyl targets (**2**, X = CH_2) were synthesized using an Evans' asymmetric Michael reaction[8] as the key step (Scheme 1).[9] Conversion of carboxylic acid **3** to the required imide **5** was accomplished using the

standard mixed anhydride method. The imide **5** was utilized in the key asymmetric Michael reaction with tert-butyl acrylate to provide the adduct **7** (de > 95%). The auxiliary was removed to yield the acid **9**, and an alkylation was performed α to the *t*-butyl ester with LDA (2 equiv) and MeI to give a 4:1 *(syn:anti)* mixture of diastereomers. The diastereomers were separated by flash chromatography of the benzyl esters, and the desired *syn* isomer was hydrogenated to the carboxylic acid **11**. In this example, **11** was coupled with the tyrosine derivative **15**. After deprotection with TFA, the desired carboxylate **17** was isolated in 50% over the last 3 steps. Compound **18** was synthesized in an analogous fashion from the biphenyl acid **4**.[10] Also synthesized was compound **19**, which differed only by the use of *n*-BuI in the alkylation step (to yield **13**). Compound **20** was synthesized by coupling the acid fluoride **14**[11] to the piperazic acid **16**[12] followed by conversion to the methyl amide. The *t*-butyl ester and the Cbz group were sequentially removed to afford the desired **20** (as shown in Scheme 1).

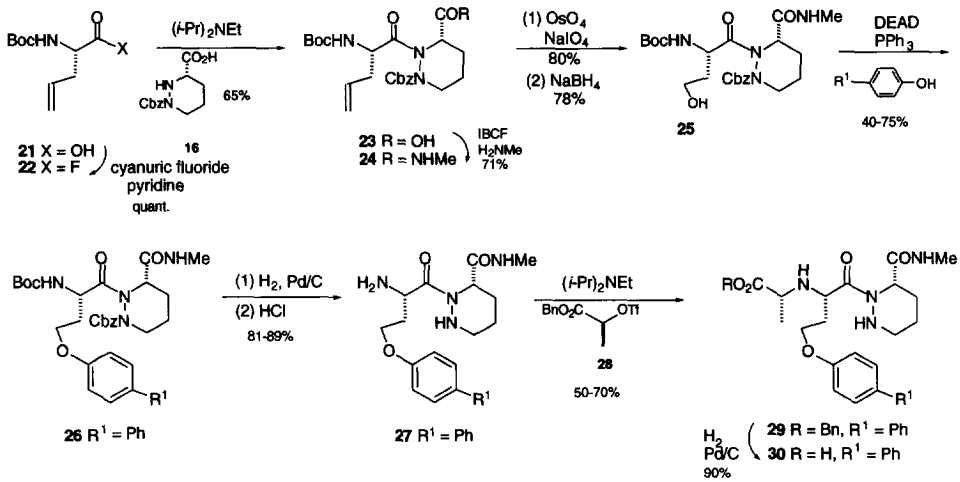
Scheme 1



The N-carboxyalkyl targets (**2**, X = NH) were synthesized from L-allylglycine as shown in Scheme 2. The N-Boc derivative **21** was converted to the acid fluoride **22** by the Carpino methodology.[11] This acid fluoride was coupled to the piperazic acid **16**, and the resulting adduct **23** was converted to its methyl amide **24** via mixed anhydride conditions. The double bond of **24** was converted to the primary alcohol **25** by an oxidative cleavage, which was followed by a $NaBH_4$ reduction. This alcohol, **25**, served as our advanced intermediate from which a

Mitsunobu reaction introduced substitution into P1'. For example, the Mitsunobu reaction between **25** and 4-phenylphenol produced the biaryl ether **26**. The Cbz and Boc groups were sequencially removed to give the primary amine **27**, which was then alkylated with the triflate **28**.[13] The resulting secondary amine **29** was hydrogenated to the desired carboxylate **30**. Other carboxylates **31**—**34** were synthesized in an analogous fashion by substitution of the appropriate phenol into the Mitsunobu reaction with **25**.

Scheme 2



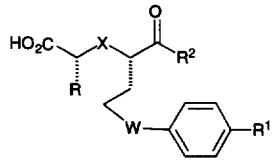
Results and Discussion

The carboxylate derivatives were tested in vitro as MMP-3 inhibitors via a MCA peptide assay as previously described.[14] The results for each compound are shown in Table 1 as K_i's.[14] From an X-ray crystal structure of pro-MMP-3,[7] we had an appreciation of the S1' pocket as being both deep and hydrophobic. We initially examined carboxylates with a phenylethyl group in P1', however as shown by compound **17** this resulted in an inactive derivative (IC_{50} = 55 μM). In order to gain additional hydrophobic affinity in the S1' pocket, we added a second phenyl ring, thus giving the biphenyl **18**. A dramatic increase in activity was observed for **18** (IC_{50} = 81 nM). This was further optimized by lengthening the P1 substituent to the n-butyl derivative **19** (K_i = 21 nM). We also desired to remove the central amide, and therefore, we synthesized the piperazic acid derivative **20**. The piperazic acid replacement was well tolerated, as **20** displayed excellent inhibition (K_i = 42 nM).

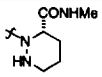
This potent biphenyl substitution in P1' was retained in the amino carboxylate series as well. The amino carboxylates were synthesized as biphenylethyl ethers in P1', thus differing in length from the above C-carboxyalkyl derivatives. Nonetheless, the parent biphenyl ether **30** (K_i = 77nM) was a very potent inhibitor of MMP-3. Compounds **31**—**34** were synthesized to probe the essential parameters of the P1' biphenyl. When a methylene spacer was placed between the two rings, as in **31**, a seven fold decrease in affinity was observed. This suggested that length and orientation of the biphenyl were critical. In an effort to replace of the pendant phenyl ring with a non-aromatic, hydrophobic group the cyclopentyl **32**, isopropyl **33**, and the *n*-butyl **34** derivatives were synthesized. Only the *n*-butyl **34** (K_i = 550 nM) showed marginal activity, as the others were

inactive. These compounds suggested that a hydrophobic group alone was not sufficient and that the π-density of the pendant ring might be essential.

Table I. In Vitro MMP-3 Inhibition



Comp. #	X	W	R	R^1	R^2	MMP-3 K_i (nM)
17	CH_2	-	Me	H	L-Tyr-(Me)-NHMe	55000 (IC_{50})
18	CH_2	-	Me	Ph	L-Tyr-(Me)-NHMe	81 (IC_{50})
19	CH_2	-	n-Bu	Ph	L-Tyr-(Me)-NHMe	21
20	CH_2	-	n-Bu	Ph	L-Ppz-NHMe	42
30	NH	O	Me	Ph	L-Ppz-NHMe	77
31	NH	O	Me	Bn	L-Ppz-NHMe	552
32	NH	O	Me	C_5H_9	L-Ppz-NHMe	>200,000 (IC_{50})
33	NH	O	Me	i-Propyl	L-Ppz-NHMe	>200,000 (IC_{50})
34	NH	O	Me	n-Butyl	L-Ppz-NHMe	550

L-Ppz-NHMe = 

Additional insight into inhibitor binding in S1' was gained from an X-ray crystal structure of MMP-3 with the biphenyl derivative **19** in the active site. As shown in Figure 1, the pendant phenyl of **19** was located at an ideal distance for an interaction[15] with the imidazole side chain of histidine 224. In fact, from our studies with the pro-enzyme,[7] it appeared that the imidazole side chain was displaced by the biphenyl. Apparently, it is this biphenyl-imidazole interaction that gives these derivatives excellent affinity for MMP-3.

Conclusions

We have identified a novel series of carboxylates as potent stromelysin inhibitors. The most active derivatives contained a biphenyl substituent in P1'. The biphenyl was employed in both C-carboxyalkyl and N-carboxyalkyl derivatives to give potent inhibitors of MMP-3. An X-ray crystal structure of inhibitor **19** bound to MMP-3 revealed an important interaction between the inhibitor's biphenyl and an active site histidine. This observation should aid in the design and development of future stromelysin inhibitors.

Figure 1. X-ray Crystal Structure of **19** Bound in MMP-3

Figure 1 Description: Active site X-ray crystal structure of inhibitor **19** (yellow carbons with blue nitrogen and red oxygen) bound to MMP-3 (gray carbons with blue nitrogen and red oxygen - catalytic zinc is orange). The imidazole side chain of histidine 224 is labeled and enlarged in order to highlight its interaction with the biphenyl of **19**.

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